Central Dopaminergic Regulation of Aldosterone Secretion in Sheep

BING-SHUAN HUANG, RICHARD L. MALVIN, JONGEUN LEE, AND ROGER J. GREKIN

SUMMARY Central dopaminergic mechanisms involved in the regulation of plasma aldosterone concentration were investigated in 16 conscious sheep following Na depletion with intramuscularly administered furosemide. Intracerebroventricular infusion of dopamine (20 μg/min) decreased plasma aldosterone significantly to 52 ± 8% of basal level and increased plasma renin activity (PRA) significantly to 172 ± 25% of basal level in this animal model. In addition, intracerebroventricular infusion of the dopamine antagonist metoclopramide (20 μg/min) in artificial cerebrospinal fluid vehicle significantly increased aldosterone levels to 144 ± 14% of basal level and decreased PRA to 62 ± 5% of basal value. Neither intracerebroventricular infusion of the vehicle nor intravenous infusions of metoclopramide or dopamine at the same doses changed aldosterone or PRA levels. Intracerebroventricular bolus injections of metoclopramide (20 μg/kg in 0.4 ml of vehicle) were also effective, increasing aldosterone levels to 266 ± 22% of basal level and decreasing PRA to 70 ± 12% of basal level. Intravenous bolus injections of the same dose of metoclopramide were ineffective. Dopamine was infused intracerebroventricularly into two uniadrenalectomized sheep with the remaining adrenal transplanted to the neck. Aldosterone levels were decreased to 49 ± 10% of basal level, and PRA was increased to 157 ± 10% of basal value. None of the infusions or injections changed arterial or intracranial pressure, or plasma K, Na, and cortisol levels. These data indicate that endogenous or exogenous dopamine may act on central dopamine receptors to decrease plasma aldosterone concentration by an unknown humoral mechanism. The known aldosterone regulators, plasma Na, K, angiotensin II, and adrenocorticotropic hormone, are not involved in the regulation.

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KEY WORDS • brain dopaminergic pathway • aldosterone secretion • adrenal transplant • metoclopramide

In earlier studies, we showed that centrally administered dopamine inhibited the release of aldosterone, an effect that is independent of changes in plasma Na, K, adrenocorticotropic hormone (ACTH), and angiotensin II (ANG II) levels. However, these studies did not clarify whether the effects of centrally infused dopamine were due to central or to peripheral actions or the mechanism by which central dopamine might suppress aldosterone secretion. In the last decade, many investigators have suggested that dopaminergic mechanisms may modulate aldosterone secretion. Some controversy exists concerning the actual role of dopamine in the regulation of aldosterone secretion and the receptor sites for dopamine in this regulation. In most in vitro studies, supraphysiological concentrations of dopamine were required to depress stimulated aldosterone secretion. The dopamine antagonist metoclopramide was reported to inhibit aldosterone biosynthesis in vitro. On the other hand, intravenous injection of metoclopramide consistently stimulates aldosterone secretion. More recently, it has been shown that infusion of dopamine or metoclopramide into the adrenal artery of sheep with adrenal autotransplants failed to change aldosterone secretion. Thus, a central mode of action of dopamine rather than a direct one on the adrenal cortex has been proposed. This study was designed to determine 1) whether central administra-
tion of a low dose of metoclopramide elicits an aldosterone response opposite to that of centrally administered dopamine, and 2) whether centrally administered dopamine and metoclopramide affect aldosterone secretion through a peripheral action. We further sought to examine whether the aldosterone response to centrally administered dopamine can be elicited in sheep with a denervated adrenal gland.

Materials and Methods

Experiments were performed in 16 conscious Ovis aries sheep, weighing between 40 and 60 kg. (The sheep were obtained from local sheep auctions.) They were housed in cages and had free access to commercial sheep pellets, hay, and water. Implantation of a permanent cannula in the lateral cerebral ventricle and construction of a skin loop in the neck containing segments of carotid artery and jugular vein were performed as described previously. Adrenal autotransplantation was performed in two sheep. Anesthesia was induced intravenously with thiopental sodium and maintained by cyclopropane through an endotracheal tube. The method of McDonald et al. was used to dissect the left adrenal gland so that its only attachment was to the renal artery and vein. Following nephrectomy, the adrenal gland, attached by its artery and vein to the segments of renal artery and vein, was transferred to the prepared neck skin loop and was transplanted by anastomosing the renal artery to the carotid artery, and renal vein to the jugular vein. A right adrenalectomy was performed on the same sheep 1 week after transplant. The viability of the transplanted gland of one sheep was confirmed by ACTH stimulation. The other was confirmed at autopsy after it died in an accident 1 month after the final operation. Preoperative and postoperative care were supervised by in-house veterinarians. Experiments commenced a minimum of 2 weeks after the completion of the operations.

To enhance adrenal sensitivity, all animals were Na-depleted by intramuscular administration of furosemide and 2 days of fasting, as previously described. All the experiments in the present study were performed at the same time of day, between 0800 and 1600. During the experiment, the sheep were loosely restrained in a sling. Arterial and intracranial pressures were recorded continuously (Grass Model 27B polygraph; Quincy, MA, USA) using catheters that had been either positioned into the carotid artery or connected to the intracerebroventricular (i.c.v.) cannula. The animals were divided randomly into six groups. A 7-hour experimental protocol was used on four groups. Artificial cerebrospinal fluid (ACSF) was infused i.c.v. or i.v. for the first 2 hours, and three blood samples were collected at 0.5-hour intervals to measure the basal hormone levels. Different drugs, dissolved in ACSF, were then infused i.c.v. or i.v. for the next 3 hours, and five blood samples were taken at 0.5-hour intervals, from 1 hour following the initiation of drug infusion to termination of drug infusion. Finally, ACSF alone was infused again for 2 hours, and three more samples were taken at 0.5-hour intervals. One of the following substances was infused during the experimental period: 1) dopamine, 20 µg/min i.c.v.; 2) dopamine, 20 µg/min i.v.; 3) metoclopramide, 20 µg/min i.c.v.; or 4) metoclopramide, 20 µg/min i.v. The infusion rate in each experiment was 40 µl/min.

An i.c.v. or i.v. bolus injection of metoclopramide (20 µg/kg in 0.4 ml of ACSF) was given in two other groups. Two blood samples were taken at 0.5-hour intervals as control. Then, 0.4 ml of ACSF was injected, and the third sample was taken 0.5 hour after injection. Finally, metoclopramide was injected, and the fourth sample was taken 0.5 hour after the injection. None of the protocols was repeated in the same animal. An i.c.v. infusion of dopamine (20 µg/min) was also given to two sheep with adrenal transplants. One sheep was used twice, and the other was used once. Blood samples were taken according to the 7-hour protocol.

Dopamine and metoclopramide were obtained from Sigma Chemical Company (St. Louis, MO, USA), and ACSF was obtained from the University of Michigan Hospital Pharmacy. There were no significant differences in pH and osmolality between the ACSF alone and ACSF with dopamine or metoclopramide.

Plasma Na and K concentrations were measured by flame photometry. Plasma aldosterone, cortisol, and renin activity (PRA) were measured by radioimmunoassay as previously described. New England Nuclear kits (Boston, MA, USA) were used for cortisol assays.

Group profile tests (multivariable Hotelling's square test) as described earlier were employed as the major statistical analysis, in which changes in plasma hormone levels are represented as the mean ± SE expressed as a difference from the basal level. This value was obtained by subtracting each of the absolute values from the basal value. In addition, Student's t test and paired t test were used to compare the basal hormone levels between groups and the levels of pretreatment and posttreatment within the same group.

Results

Intact Sheep

As shown in Table 1, there were no significant changes in mean arterial blood pressure or plasma K, Na, and cortisol levels in any of the experimental groups following any of the injections or infusions. There were no significant changes in intracranial pressure.

The response of plasma aldosterone and PRA to i.c.v. dopamine infusion (20 µg/min) are shown in Figure 1A. The data (n = 8) include six animals for which data were presented in a previous paper. As shown, i.c.v. dopamine infusion decreased aldosterone levels significantly to 52 ± 8% of basal level. The levels remained low as long as 1.5 hours after the termination of dopamine infusion. The PRA responses were actually opposite to those of aldosterone, increasing significantly to 172 ± 25% of basal value.

The responses to i.c.v. metoclopramide infusion (20
TABLE 1. Plasma Na, K, and Cortisol Levels and Mean Arterial Blood Pressure of all Animals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plasma Na (mEq/L)</th>
<th>Plasma K (mEq/L)</th>
<th>Cortisol (ng/ml)</th>
<th>Mean arterial pressure (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal level</td>
<td>After infusion</td>
<td>Basal level</td>
<td>After infusion</td>
</tr>
<tr>
<td>ACSF, i.c.v. (n = 5)</td>
<td>132 ± 2</td>
<td>131 ± 2</td>
<td>3.8 ± 0.3</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>Dopamine, i.c.v. (n = 5)</td>
<td>135 ± 3</td>
<td>134 ± 2</td>
<td>3.9 ± 0.3</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>Dopamine, i.v. (n = 4)</td>
<td>129 ± 7</td>
<td>130 ± 5</td>
<td>3.6 ± 0.4</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>MCP, i.c.v. (n = 7)</td>
<td>129 ± 6</td>
<td>130 ± 7</td>
<td>3.7 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>MCP, i.v. (n = 4)</td>
<td>130 ± 8</td>
<td>129 ± 9</td>
<td>3.8 ± 0.7</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>MCP, i.c.v. bolus (n = 7)</td>
<td>130 ± 4</td>
<td>130 ± 6</td>
<td>3.7 ± 0.3</td>
<td>3.7 ± 0.5</td>
</tr>
<tr>
<td>ACSF, i.c.v. bolus (n = 7)</td>
<td>130 ± 4</td>
<td>131 ± 3</td>
<td>3.7 ± 0.3</td>
<td>3.6 ± 0.7</td>
</tr>
<tr>
<td>MCP, i.v. bolus (n = 5)</td>
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<td>133 ± 7</td>
<td>3.6 ± 0.2</td>
<td>3.6 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. Each postinfusion value is the mean of five samples taken at 0.5-hour intervals following the initiation of drug infusion. (None of these five values was significantly different from basal value.)

ACSF = artificial cerebrospinal fluid; MCP = metoclopramide.

Figure 1. Aldosterone and PRA responses to i.c.v. infusion of dopamine (A) in eight sheep and metoclopramide (MCP; B) in seven sheep. Each point is the mean ± SE. The drugs were infused from 0 to 3 hours. Asterisk indicates the p value is less than 0.05 by paired t test, when each point was compared with the mean basal level.

μg/min) are shown in Figure 1B. The i.c.v. metoclopramide infusion increased aldosterone levels significantly to 146 ± 10% of basal level, whereas PRA decreased significantly to 62 ± 5% of basal value. Following termination of the metoclopramide infusion, aldosterone and PRA levels returned toward basal levels.

The responses of aldosterone and PRA to i.v. dopamine or metoclopramide infusions are shown in Figure 2. We have shown in an earlier work that i.c.v. ACSF infusion has no effect on PRA or aldosterone levels. For comparison those data are presented again in this figure. As shown, there were no significant changes in aldosterone and PRA levels following any of these infusions.

Profile tests for the changes of aldosterone level showed significant group differences between the i.c.v. dopamine and i.c.v. ACSF infusion groups (p = 0.008), i.c.v. dopamine and i.v. dopamine infusion groups (p = 0.005), i.c.v. metoclopramide and i.c.v. ACSF infusion groups (p = 0.03), and i.c.v. and i.v. metoclopramide infusion groups (p = 0.04). Profile tests for the changes in PRA level showed significant differences for the same group comparisons (p = 0.04, 0.02, 0.03, and 0.007, respectively). There were no significant differences of basal aldosterone or PRA levels between any of the groups.

The results of the bolus injections are shown in Figure 3. Again, i.c.v. metoclopramide injection increased aldosterone levels but reduced PRA. No significant effects were seen with any of the other injections.

Adrenal Transplants

No evidence of adrenal insufficiency was observed in the sheep with an adrenal transplant. They continued
to gain weight following operation and appeared normal in all respects. An i.v. bolus injection of ACTH (5 U) in one sheep increased plasma aldosterone level to 296% of basal value 30 minutes after injection. Pathological examination of the other sheep indicated that the transplanted gland was vital and the vessel anastomoses were patent. The aldosterone and PRA responses to i.c.v. dopamine infusion (20 μg/min) in these two animals are plotted individually in Figure 4. During i.c.v. dopamine infusion, the plasma aldosterone level in these sheep decreased by approximately 50% and PRA increased to 157% of basal values 2 hours after infusion. With the sample size of three sheep, these responses were statistically significant when they were compared with the basal aldosterone and PRA levels (p = 0.02 and 0.04, respectively) and were similar to the aldosterone and PRA responses to i.c.v. dopamine infusion at the same dose in intact sheep (52 ± 8% and 172 ± 25% of basal levels, respectively).

Discussion

No behavioral changes were observed in the sheep during any of the experiments. The lack of changes in plasma Na, K, and cortisol levels after infusion of dopamine or metoclopramide indicates that changes in these variables are unlikely to be responsible for the changes in aldosterone secretion. Involvement of baroreceptor reflexes in the response is also unlikely, since arterial and intracranial pressures were stable throughout. PRA was increased by i.c.v. administration of dopamine and decreased by i.c.v. administration of metoclopramide. Since PRA and plasma aldosterone levels changed in opposite directions, the aldosterone responses to dopamine and metoclopramide in this study appear to be largely independent of changes in the plasma ANG II concentration. The changes in PRA following i.c.v. dopamine or metoclopramide administration in the present study are quite different from other reports, in which i.v. administration of dopamine or metoclopramide did not alter the PRA in human subjects. We demonstrated previously that i.c.v. administered dopamine significantly decreased plasma levels of antidiuretic hormone (ADH) in sheep.1 It is commonly accepted that ADH depresses renin secretion and may play an intermediary role in regulating PRA levels.15 Thus, we believe that the changes in PRA in the present study are secondary to the changes in ADH levels. It is not clear whether i.v. administered metoclopramide at higher doses has a direct effect on renin secretion.
adrenergic receptor antagonist phentolamine or the \( \beta \)-
dopamine was attenuated by pretreatment with i.c.v.
the blood pressure of dogs. This central effect of
dopamine administered i.c.v. significantly changed
central dopamine receptors. As a precursor of norepi-
phrine, centrally injected dopamine may also modu-
late aldosterone and renin secretion through the sym-
pathetic system. Lang and Woodman observed that
injection of dopamine antagonists, but not by the \( \alpha \)-
adrenergic receptor antagonist propranolol. On the
other hand, Wilson et al. demonstrated that, after pre-
treatment with the ganglionic blocker trimethaphan,
the aldosterone responses to metoclopramide remained
intact in human subjects but were abolished in sheep.
They suggested that dopaminergic inhibition of aldos-
sterone secretion is independent of the autonomic ner-
vous system in humans but not in sheep. The present
study, however, provides evidence that centrally in-
fluenced dopamine depresses aldosterone secretion in
sheep with a single denervated adrenal gland. This
finding supports the hypothesis that centrally adminis-
tered dopamine regulates hormones and blood pressure
through central dopaminergic receptors and that this
regulation is independent of autonomic innervation of
the adrenal gland. Central dopaminergic pathways
may tonically inhibit release of an aldosterone stimu-
lating hormone.

Many in vivo studies have shown that i.v. injection
of dopamine agonists and antagonists significantly
changes aldosterone secretion. In these studies, a
10-mg i.v. dose of metoclopramide was necessary to
increase plasma aldosterone levels significantly in
humans and dog. We examined the aldosterone
responses to i.v. or i.c.v. metoclopramide admin-
istration following a bolus injection using a dose ap-
proximately 10 times lower than that of other stud-
ies. The i.c.v. injection significantly decreased
plasma aldosterone levels, whereas the i.v. injection
had no effect. The likely explanation is that only when
the blood concentration of metoclopramide is high
enough to reach central receptors, can the aldosterone
stimulating effects be expressed. In contrast to these
observations are studies both in vivo and in vitro showing
that dopamine has direct effects on aldosterone
secretion. Drake et al. showed that dopamine, 4
\( \mu g/kg/min \) i.v., decreased aldosterone responses to
ANG II in human subjects. Since dopamine is unable
to cross the blood-brain barrier in physiological con-
centrations, it appears that there are two dopaminer-
gic mechanisms that may affect aldosterone secretion.
One pathway exists peripherally within the adrenal and
mediates the response to i.v. infusion of dopamine, its
agonists, and its antagonists; the other is located in the
central nervous system and is only expressed when
centrally active compounds are administered.

McDougall et al. and Lun et al. infused dopamine
or metoclopramide directly into the adrenal artery of
sheep with one adrenal gland autotransplanted to a skin
loop in the neck. They found that direct infusions
failed to affect aldosterone secretion in these sheep.
Since systemically administered metoclopramide stim-
ulated aldosterone secretion in the same animals, they
concluded that local (adrenal) dopaminergic mecha-
nisms play little or no part in the regulation of aldoste-
ronate secretion in sheep. They speculated that dopa-
mine acted centrally. Their observations are consistent
with the results of the present study, which provides
direct evidence that the dopaminergic effect on aldos-
sterone secretion does not occur through a direct action
on the adrenal cortex or other peripheral receptors.
Plasma aldosterone levels were significantly changed

Metoclopramide has been shown to be a specific
agonist of dopamine in the central nervous sys-
tem. It antagonizes the inhibitory effect of dopamine
on prolactin release. In addition to the finding that
metoclopramide increases aldosterone secretion in
vivo, evidence has been provided to show that dopa-
mine blocks metoclopramide-stimulated aldosterone
secretion in a dose-dependent fashion. Therefore, it is reasonable to conclude that the aldosterone
response to metoclopramide is mediated by metoclo-
pramide’s antagonist activity at the level of the dopa-
mine receptor.

In the present study, i.c.v. administration of meto-
clopramide elicited aldosterone and PRA responses
that were qualitatively opposite to those produced by
i.c.v. dopamine infusion. The implications are that 1) endogenous brain dopamine physiologically modu-
lates aldosterone and renin secretion by some unknown
mechanism, and 2) dopamine exerts its effect through
central dopamine receptors. As a precursor of norepi-
nephine, centrally injected dopamine may also modu-
late aldosterone and renin secretion through the sym-
pathetic system. Lang and Woodman observed that
dopamine administered i.c.v. significantly changed
the blood pressure of dogs. This central effect of
dopamine was attenuated by pretreatment with i.c.v.
injection of dopamine antagonists, but not by the \( \alpha \)-
adrenergic receptor antagonist phentolamine or the \( \beta \)-

FIGURE 4. Aldosterone (A) and PRA (B) responses to i.c.v.
infusion of dopamine in two sheep with transplanted adrenals.
The same experiment was performed twice on Sheep 2 on differ-
ent dates, and the data were plotted separately, labeled as Sheep 2a and Sheep 2b.
by i.c.v. dopamine or metoclopramide infusions, but not by i.v. infusions of the same substances.

Of interest are the species differences with regard to dopaminergic regulation of aldosterone secretion. Although many investigators have shown that peripherally infused dopamine or metoclopramide alters aldosterone secretion in humans,3, 13, 14, 20 dopamine reportedly had no effect on aldosterone output of bovine adrenal cells, nor did it block the aldosterone response to ANG II in superfused rat adrenal cells.9 In addition to the present observation, studies on Na-depleted or Na-replete sheep showed that dopamine or metoclopramide had basically no direct effect on the adrenal.8, 10 Furthermore, metoclopramide had no effect on plasma corticosteroid levels in humans,3, 13, 14 but it stimulated corticosteroid secretion in sheep.5, 24 Metoclopramide was shown to stimulate aldosterone secretion in sheep by a mechanism related to the autonomic nervous system,25 but the involvement of the autonomic system in dopaminergic regulation of aldosterone secretion was excluded in humans.3 Clearly, further studies are required to determine whether species differences are crucial in explaining the contrary observations.

Dopamine has been demonstrated to be an important central neurotransmitter that acts as a regulator of pituitary hormone secretion through the tuberoinfundibular system.26 Although i.c.v. infusion of dopamine or metoclopramide did not affect ACTH secretion, a pituitary factor other than ACTH may mediate dopaminergic effects on aldosterone secretion. Pituitary hormones known to affect aldosterone secretion include \( \alpha \)-melanotropin,27, 28 \( \beta \)-lipotropin,29 \( \beta \)-melanotropin,28 and aldosterone stimulating factors.30, 31 In summary, centrally administered dopamine and metoclopramide, in doses that are ineffective intravenously, alter plasma aldosterone levels in opposite directions in Na-depleted sheep. The changes did not appear to be due to changes in ANG II, as PRA increased as aldosterone fell and vice versa. Centrally administered dopamine decreased aldosterone secretion even after the denervation of the adrenal. These results offer support for the possible role of a central dopaminergic system in the regulation of aldosterone secretion in Na-depleted sheep and imply that the effector limb of the system is hormonally mediated.

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